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Seasonal ectomycorrhizal fungal biomass development on loblolly pine (*Pinus taeda* L.) seedlings

Abstract Ergosterol, a membrane sterol found in fungi but not in plants, was used to estimate live mycelial biomass in ectomycorrhizae. Loblolly pine (*Pinus taeda* L.) seeds were sown in April 1993 and grown with standard nursery culture practices. Correlations between total seedling ergosterol and visual assessment of mycorrhizal colonization were high during July and August but low as ectomycorrhizal development continued into the growing season. Percentages of mycelial dry weight over lateral roots decreased from 9% in July to 2.5% in November because seedling lateral root dry weight accumulated faster than mycelial dry weight. Total ergosterol per seedling increased from July through February. As lateral root dry weight ceased to increase during winter months, ectomycorrhizal mycelia became the major carbohydrate sink of pine seedlings. No distinctive seasonal pattern of soil ergosterol content was observed. The impact of ectomycorrhizal fungi on plant carbohydrate source-sink dynamics can be quantitatively estimated with ergosterol analysis but not with conventional visual determination.

Key words Ergosterol · Loblolly pine · Seasonal · Ectomycorrhizal fungal biomass

Introduction

In 1993, over 1 billion seedlings were produced by nurseries in the southern United States (USDA Forest Service 1993). Most nursery-grown seedlings in this region are either colonized with naturally occurring ectomycorrhizal fungi or artificially inoculated with specific species (Marx et al. 1991). Numerous studies on the beneficial effects of ectomycorrhizal fungi on seedling growth and development in artificially regenerated forest sites and drastically disturbed land have been conducted (Marx et al. 1991 and references cited therein). In these studies, the extent of mycorrhizal colonization is often assessed visually, mostly by determining percentages of short roots colonized and numbers of ectomycorrhizal roots per unit length of lateral roots. Recently, Marx (1990) developed a more detailed visual assessment system based on the morphological types of the mycorrhizae to account for the various degrees of mycorrhizal proliferation from roots with the same percentage of short root colonized. Such a system attempts to indirectly relate visual morphological estimates of mycorrhizal colonization to fungal biomass.

Analytical chemical techniques are increasingly used to estimate mycorrhizal fungal biomass. Specifically, analyses of ergosterol and chitin have become popular alternatives to the traditional visual assessments. Ergosterol is the main fungal sterol and an important component of the mycelial membranes except in the Oomycete genera, *Pythium* and *Phytophthora* (Weete 1980; Mercer 1984). Because plants do not contain ergosterol and because ergosterol is only present in living mycelia, a chemical analysis of ergosterol from roots of mycorrhizal plants can be used to quantify the degree of mycorrhizal colonization.

Salmanowicz and Nylund (1988) reported the first use of ergosterol analysis to quantify ectomycorrhizal fungal biomass in a study of *Pinus sylvestris* L. seedlings. Since then, ergosterol has been analyzed in several studies of ectomycorrhizal *Pinus sylvestris* (Nylund and Wallander 1989; Wallander and Nylund 1991; Antibus and Sinsabaugh 1993), *Pinus contorta* Dougl. ex. Loud (Johnson and McGill 1990a), *Eucalyptus globulus* Labill. (Martin et al. 1990), and *Betula papyrifera* Marsh. (Antibus and Sinsabaugh 1993) and in studies of vesicular-arbuscular mycorrhizal tree species (Schmitz et al. 1991; Frey et al. 1992; Antibus and Sinsabaugh 1993).

In contrast, chitin analysis may not provide reliable estimates of fungal biomass. Unlike ergosterol, chitin is

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present in both living and dead mycelia. Furthermore, analysis of chitin is neither as simple nor as sensitive as analysis of ergosterol (Martin et al. 1990).

In mycorrhizal fungus-root symbiosis, fungal mycelia exchange inorganic nutrients for plant carbohydrates. Experiments conducted on *Pinus ponderosa* Dougl. ex Laws seedlings colonized by *Hebeloma crustiliniforme* (Bull. ex Fr.) Quel. indicated the fungus received 7% of the total ^{14}C fixed (Rygiewicz and Andersen 1994). In many plants, including loblolly pine, sucrose is the major form of translocated carbohydrate (Shiroya et al. 1962). Sung et al. (1993) reported that loblolly pine roots have three soluble sucrose-cleaving enzymes, namely sucrose synthase, acid invertase, and alkaline invertase, to metabolize sucrose for respiration, growth, and storage. No cell wall-bound invertase was found in ectomycorrhizal fungi (Salzer and Hager 1991). Ectomycorrhizal fungi may utilize hexoses supplied by roots after sucrose hydrolysis by root cell wall acid invertase (Taber and Taber 1987; Salzer and Hager 1991; Chen and Hampp 1993). Therefore, analyses of sucrose-cleaving enzymes provide estimates of source-sink relationships and carbon allocation pattern in forest trees.

Sung et al. (1993) reported seasonal patterns of sucrose metabolism for loblolly pine seedlings in relation to source-sink activity but did not determine the seasonal carbon sink activity attributable to ectomycorrhizal fungi on nursery-grown loblolly pine seedlings. The objectives of this study were: (1) to assess the seasonal development of ectomycorrhizal fungal biomass on loblolly pine seedlings using ergosterol analysis, (2) to evaluate the visual assessment method developed by Marx (1990), and (3) to provide information on the seasonal source-sink relationships of loblolly pine seedlings.

Materials and methods

Preliminary study

To work out protocols for seedling sampling and ergosterol extraction procedures, first-year loblolly pine, slash pine (*Pinus elliottii* Engelm.), and longleaf pine (*Pinus palustris* Miller) seedlings were sampled from various commercial nurseries located in the southern United States. Ergosterol analysis was performed from individual lateral roots of the seedlings. In 1994, a 9-year-old loblolly pine tree, grown in a Georgia nursery, was excavated with a 1.3-m-diameter tree spade and fine roots were collected for ergosterol analysis.

Seedling growth

Sixty $0.6 \times 0.6 \times 0.6$ m above-ground plywood microplots, located at the Whitehall Nursery, Athens, Ga., were filled with a methyl bromide-fumigated soil mixture, at a ratio of 2 parts forest soil (sandy clay loam Hapludult) to 1 part sand and 1 part milled pine bark. In mid-April 1993, 20 g pulverized 10-10-10 fertilizer (560 kg/ha) was mixed into the upper 10 cm of soil in each plot. Each plot was then sown with 100 stratified loblolly pine seeds. All seedlings were fertilized with 56 kg N/ha as NH_4NO_3 (6.5 g/plot) in early June, July, August, and September. Seedlings were hand watered at least three times a week and daily during summer.

Seedling sampling and analysis

From July 1993 through May 1994, loblolly pine seedlings were carefully excavated every month from six randomly selected microplots. Five seedlings randomly selected from each plot were visually and chemically assessed for mycorrhizal development. Within 1 h of lifting, visual assessment was made on each seedling according to Marx's (1990) rating system. Besides the percentage of short root colonized, the degree of morphological proliferation for colonized short roots was also rated. Immediately after visual assessment, individual seedlings were dissected into needles, stem and branches, tap root, and first order lateral roots. Oven dry weight (dry wt.) was obtained for all seedling parts except lateral roots. Lateral roots were freeze dried at $-55°C$ for 24 h, weighed, and processed for ergosterol without delay. By November, the mass of seedling root systems had increased substantially. For this reason, only the second and higher order lateral roots stripped from the first order lateral roots were freeze dried and used for ergosterol analysis.

At each sampling, after the seedlings had been lifted, 1 l of soil was taken from the top 25 cm of each of the six microplots. The soil was quick dried in an oven for 2 h at 60° C, screened through 2.0-mm and 0.841-mm mesh sieves, stored at $-20°C$ overnight, and analyzed for ergosterol within 24 h. Control tests showed that 2-h quick drying of soil at 60° C did not affect the ergosterol content of pure *Pisolithus tinctorius* (Pers.) Coker & Couch mycelia amended in the soil samples.

Ectomycorrhizal fungal cultures

Pure ectomycorrhizal fungal cultures were used to construct standard curves for live mycelial dry wt. versus ergosterol content. Several commonly occurring mycorrhizal fungal species in the southern United States were grown at 25° C in 250-ml flasks containing 80 ml modified Melin-Norkrans (MMN) liquid medium for 40 days. Mycelia were harvested by vacuum filtration, rinsed with deionized water, freeze dried, and analyzed for ergosterol. There were five replicates for each species. The species used were *Amanita muscaria* (Pers. ex Fr.) Hock. (isolate no. 159) (Am), *Cenococcum geophilum* Fr. (no. 146) (Cg), *Hebeloma crustuliniforme* (no. 285) (Hc), *Laccaria laccata* (Scop. ex Fr.) Berk & Br. (n. 87) (Ll), *Pisolithus tinctorius* (no. 311) (Pt), *Paxillus involutus* Batsch ex Fr. (no. 312) (Pi), *Rhizopogon luteolus* (no. VT 2394) (Rl), *Suillus luteus* (L. ex Fr.) S. F. Gray (no. 92) (Sl), and *Thelephora terrestris* (Ehrh.) Fr. (no. 35) (Tt). In more detailed tests, Pt (no. 311), *Rhizopogon* sp. (no. 173) (Rh), and Tt (no. 35) were grown at 25° C in 100 ml MMN liquid for 75–96 days and sampled periodically. Each time, four flasks of mycelia were harvested and analyzed separately for each species.

Ergosterol extraction from roots and fungi

Procedures for chemical extraction and HPLC determination of ergosterol from seedling roots and fungal cultures were adapted from Nylund and Wallander (1992). A series of control tests, such as recovery of added authentic ergosterol or ectomycorrhizal fungal mycelia to plant root samples, before and after freeze drying, were performed. In these control tests, the recovery of ergosterol was at least 92%. Because colonization among lateral roots of the same seedling varies, ergosterol was extracted from the entire lateral root system, or from all the stripped fine roots, of each seedling. Tissues were ground in liquid N_2 with a pestle and mortar. The powdered tissues were further homogenized in 8–32 ml 95% ethanol containing 2 mM dithiothreitol (DTT). The resulting homogenate was poured into a 50-ml centrifuge tube. A 60% (w/v) KOH solution was added to give a final concentration of 10% KOH (w/v). Samples were centrifuged for 10 min at 16000 rpm at 20° C and supernatant volume was recorded. An aliquot of 9.5 ml supernatant was transferred to a 25-ml screw-cap tube containing a few boiling chips and saponified for 30 min in a heating block at

100° C. Deionized water (1 ml) and *n*-hexane (5 ml) were added to each of the tubes after cooling to room temperature. Tubes were then vortexed vigorously for 1 min. The upper hexane layer containing ergosterol was quantitatively transferred to another tube. Ergosterol remaining in the lower aqueous layer was further extracted twice using 5 ml *n*-hexane each time. The combined hexane solution was dried in a heating block at 40° C under a gentle stream of N_2 gas. After cooling, 1–5 ml methanol was added to the tubes, which were then returned to the heating block to dissolve the residue at 40° C. The methanol extract was filtered with a 0.45-μm syringe filter before HPLC analysis.

HPLC determination

A Dionex 4500i HPLC system was used for ergosterol analysis. A 4.6 mm × 12.5 cm Zorbax reverse C-18 column, with a sample loop of 25 μl was used. The eluent was 100% methanol at a flow rate of 1 ml/min. The UV detector was set at 282 nm. The retention time for ergosterol was 4.6 min. The ergosterol peak from the mycelial, root, and soil samples was verified by spiking the extracts with a known amount of authentic ergosterol. For every 8–10 samples, a set of ergosterol standards of known concentrations was used as external standards. The linear portion of the ergosterol standard curve was between 1 μg/ml and 80 μg/ml with a 0.5 μg/ml detection limit.

Ergosterol extraction from soil and HPLC determination

Procedures for chemical extraction of ergosterol from soil samples were adapted from Davis and Lamar (1992). Screened soil (50 g) was placed in a 250-ml beaker and stirred for 2.5 h in a 50-ml ethanol-DTT extraction solution containing 10 ml 60% (w/v) KOH. The extract was decanted into a 250-ml centrifuge bottle using an additional 10 ml extraction solution to rinse the beaker. The solution was then centrifuged at 20° C for 10 min at 10000 rpm. The supernatant volume was measured and an aliquot of 9.5 ml was placed in a 25-ml screw-cap culture tube with several boiling chips. The extracts were processed further using the procedures described in the previous section. Ergosterol recovery tests and peak verification on the chromatogram for soil were the same as those used for root samples. Soil recovery of authentic ergosterol was 88%.

Results

Ectomycorrhizal fungal cultures

For Tt, the developmental patterns of mycelial dry wt. and total mycelial ergosterol were similar (Fig. 1a, b). A lag period was observed in both parameters for the first 40 days of incubation. A period of linear increases in ergosterol and dry wt. followed for at least 3 weeks. A twofold range in ergosterol content, from 2.5 to 5.0 μg/mg mycelial dry wt., was observed over the entire incubation period, with higher ergosterol levels in 55-day and older mycelial cultures than in 22-day and younger mycelial cultures (Fig. 1c).

Rh displayed a relationship similar to Tt between mycelial dry wt. and total ergosterol (Fig. 2a, b). No obvious lag period was observed with Rh. The most active period of growth occurred after 70 days of incubation as determined by dry wt. Ergosterol content of Rh ranged between 2 and 3 μg/mg mycelial dry wt. without any clear developmental pattern (Fig. 2c).

Pt displayed variable growth rates within an isolate. Some flasks had much slower growing colonies than others. Figure 3 presents developmental patterns from both slow- and fast-growing Pt, judged arbitrarily by its mycelial dry wt. Whenever mycelial dry wt. from a flask was less than or greater than 30% of the average of all four samples at given incubation time, this culture was designated as a slow-growing or a fast-growing culture, respectively. In some cases, all four flasks sampled had 50% or less mycelial dry wt. as compared with fast-growing cultures sampled earlier. These were identified

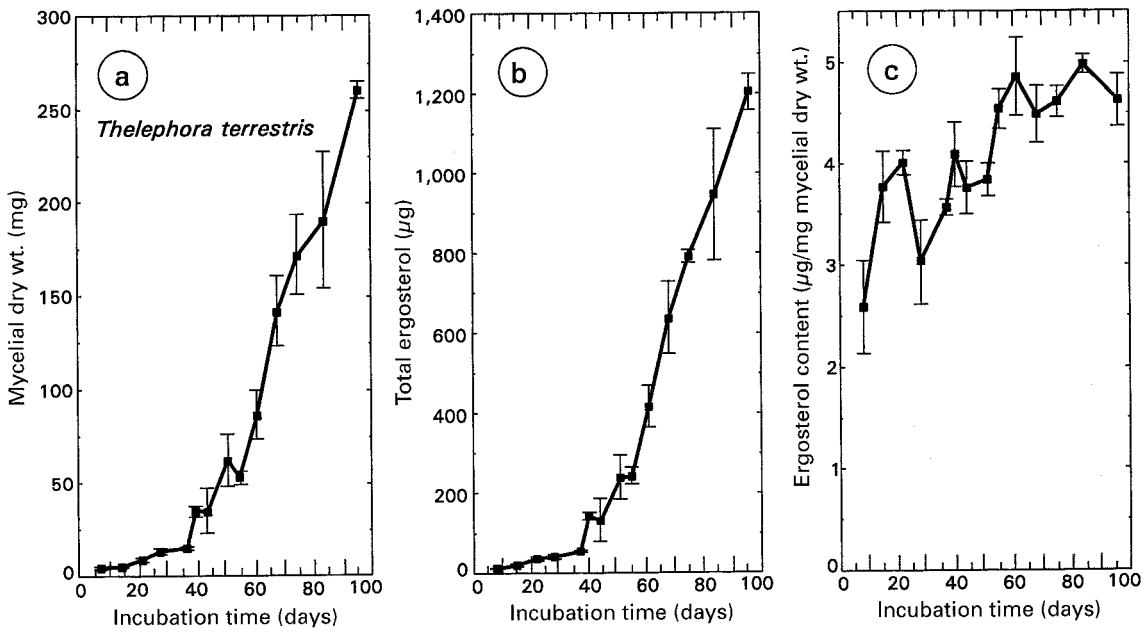

Fig. 1 Temporal patterns of **a** mycelial dry wt., **b** total ergosterol amount, and **c** ergosterol content of *Thelephora terrestris* (isolate no. 35) grown at 25° C in 100 ml liquid MMN medium for 96 days. The values presented are means ± standard deviation ($n = 4$)

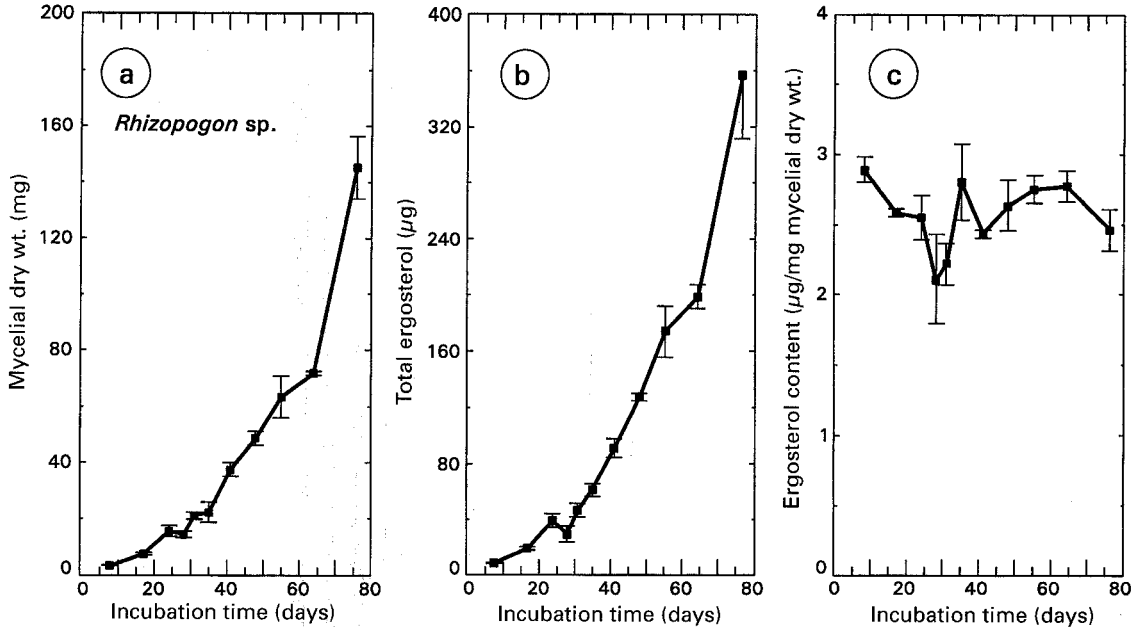

Fig. 2 Temporal patterns of **a** mycelial dry wt., **b** total ergosterol amount, and **c** ergosterol content of *Rhizopogon* spp. (isolate no. 173) grown at 25° C in 100 ml liquid MMN medium for 78 days. The values presented are means ± standard deviation ($n=4$)

as slow-growing cultures. Between 15 and 55 days of incubation, twice as much mycelial biomass was present in the fast-growing cultures compared to the slow-cultures Pt (Fig. 3a). During this time period, total ergosterol mirrored mycelial growing biomass (Fig. 3b). No apparent differences in the ergosterol content between fast and slow cultures of Pt were observed (Fig. 3c). Pt was the only ectomycorrhizal fungal species studied to exhibit decreasing ergosterol content with age (Fig. 3c).

Ergosterol contents for several other ectomycorrhizal fungal species are given in Table 1. Species such as Ll, Rl, and Tt had much smaller standard deviations in ergosterol content than Am and Hc (Table 1). Tt had similar levels of ergosterol to those in Figure 1c and Pt had ergosterol content and dry wt. equivalent to those of the slow-growing culture in Figure 3c. Rl had twice as much dry wt. at day 40 (Table 1) than that of Rh in Figure 2c. However, the identity of Rh in Figure 2 was not known. An average of 3.31 μg/mg mycelial dry wt.

Fig. 3 Temporal patterns of **a** mycelial dry wt., **b** total ergosterol amount, and **c** ergosterol content of *Pisolithus tinctorius* (isolate no. 311) grown at 25° C in 100 ml liquid MMN medium for 75 days with the fast-growing cultures (■) and for 50 days with the slow-growing cultures (□). Values presented are means ± standard deviation for both fast- and slow-growing cultures ($n=3$ or 4). Values without standard deviation are from a single sample

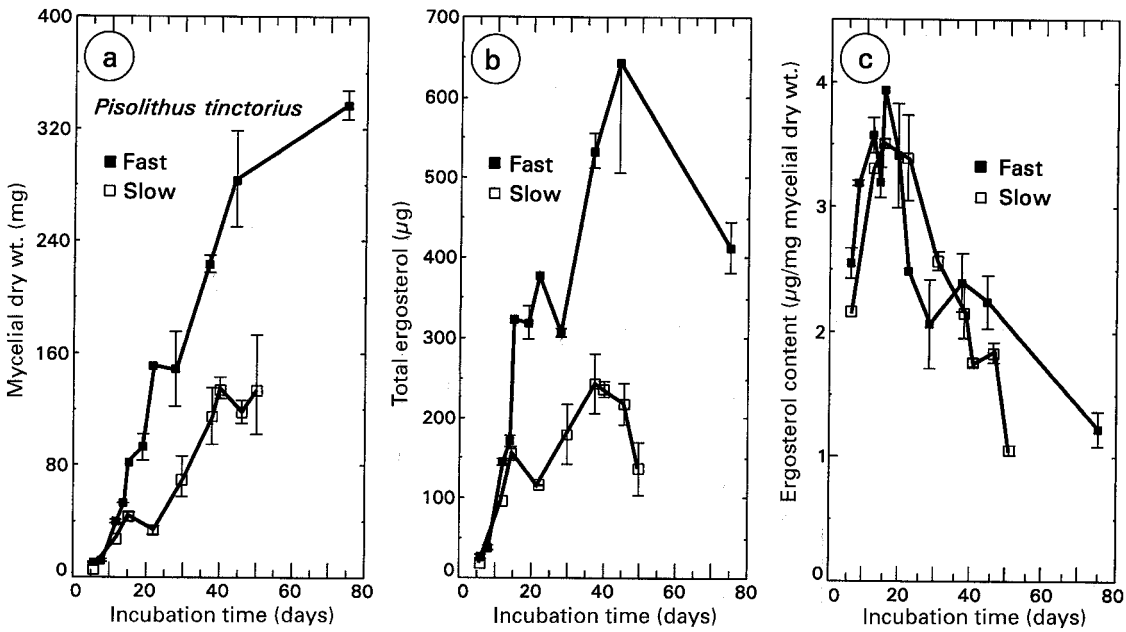

Table 1 Average ergosterol contents of ectomycorrhizal species grown at 25° C in 80 ml liquid MMN media for 40 days. Five samples were analyzed for each species and are shown as means ± standard deviation

Species	Mycelial dry wt. (mg)	Ergosterol content (μg/mg mycelial dry wt.)
Amanita muscaria	37.3±24.4	4.68±1.88
Cenococcum geophilum	55.2± 4.4	1.54±0.23
Hebeloma crustuliniforme	59.5± 4.3	3.13±1.73
Laccaria laccata	41.4± 6.1	6.55±0.31
Paxillus involutus	41.1± 5.7	6.28±1.25
Pisolithus tinctorius	126.9±23.0	2.24±0.78
Rhizopogon luteolus	191.5±33.7	7.98±0.99
Suillus luteus	60.9±33.4	3.90±0.57
Thelephora terrestris	31.9± 6.2	4.17±0.23

was obtained from data presented in Figures 1c, 2c, 3c, and Table 1. Judging from mycelial dry wt. for each species at day 40 of incubation, the fastest growing species were Rl and Pt, followed by Sl, Hc, and Cg (Table 1). The slowest growing species was Tt.

Ergosterol contents of nursery-grown southern pines

All samples from seedlings and the 9-year-old loblolly pine tree had detectable ergosterol contents. Within-group variation in ergosterol content was large (Table 2). The Pt-inoculated longleaf pine seedlings had very high levels of ergosterol (Table 2). The Pt-inoculated loblolly pine had similar levels of ergosterol to seedlings naturally colonized by mycorrhizal fungi sampled in October (Fig. 4).

Ergosterol levels of individual loblolly pine seedling grown in microplots

Total ergosterol levels generally increased from July 1993 through February 1994. Decreases in ergosterol levels were observed only in December and March (Fig. 4a). Although not significant, there was a slight and consistent decrease in ergosterol content, μg/g lateral root dry wt., from July through November. Thereafter, ergosterol content remained constant through May 1994 (Fig. 4b). Ergosterol content per g needle dry wt. did not show any clear seasonal pattern (Fig. 4c). Percentages of ectomycorrhizal fungal biomass over lateral root dry wt. were obtained with a value of 3.31 μg ergosterol/mg mycelial dry wt. (Fig. 4d). In July, 9% of the seedling lateral root biomass was from live mycorrhizal fungi. Mycelial dry wt. percentage decreased rapidly over the next 3 months and remained at about 3% through May 1994 (Fig. 4d).

Ergosterol levels in microplot soil

Soil ergosterol levels ranged between 150 μg/l for July and 750 μg/l for May (Table 3). No seasonal patterns in soil ergosterol levels were observed. Large variations in ergosterol levels occurred in each sampling period. The derivation of mycelial dry wt. in Table 3, expressed as milligrams per liter of soil, was based on two assumptions: (1) ergosterol found in the soil samples was attributed completely to extramatrical mycorrhizal mycelia, and (2) ergosterol per mg mycelial dry wt. was 3.31 μg.

Visual assessments of mycorrhizal colonization

The percentage of mycorrhizal colonization of microplot-grown loblolly pine seedlings doubled from July to August and remained constant through November (Table 4). Colonization percentage decreased slightly in December and then doubled by February 1994. No clear seasonal pattern was observed with the morphological rating of mycorrhizae (Table 4). The lowest and highest morphological rating occurred in July and October, respectively. To determine the correlations between visual and chemical assessments of mycorrhi-

Table 2 Analysis of ergosterol from southern pines grown in various commercial nurseries located in the southern United States. Data shown are means ± standard deviation (Pt *Pisolithus tinctorius*)

Host plant	Age (months)	Nursery location	Sampling date	Ergosterol content	*n*
				μg/cm lateral roots	
Pinus taeda	6	Georgia	Sept 92	0.76± 0.63	36
Pinus palustris	10	Georgia	Sept 92	0.43± 0.29	19
Pinus elliottii	6	Georgia	Sept 92	0.83± 1.02	27
				μg/g lateral root fresh wt.	
Pinus taeda	9	Alabama	Jan 93	28.8 ± 26.9	82
				μg/g lateral root dry wt.	
Pt-inoculated *Pinus palustris*	12	S. Carolina	Nov 92	400 ±156	29
Pt-inoculated *Pinus taeda*	6	Georgia	Oct 93	119 ± 59	5
				μg/g fine root dry wt.	
Pinus taeda	108	Georgia	May 94	526 ±257	7

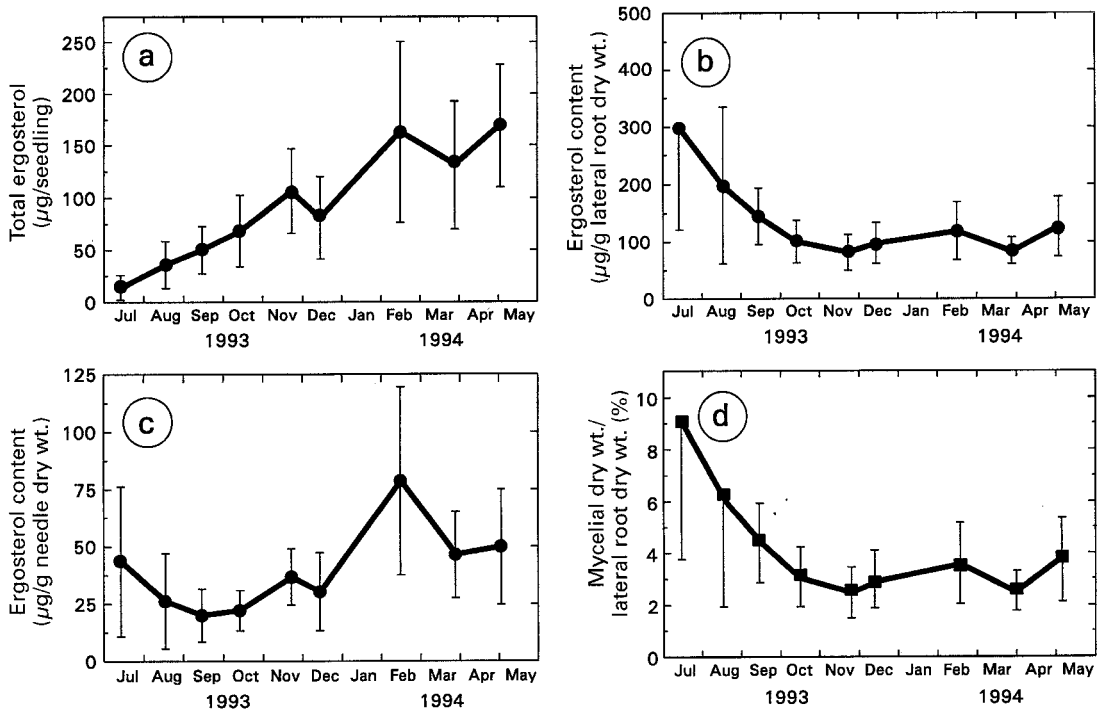

Fig. 4 Seasonal patterns of **a** total ergosterol (μg/seedling), **b, c** ergosterol contents, and **d** ectomycorrhizal fungal biomass as a percentage of lateral root dry wt. in loblolly pine seedlings from the microplots, calculated using the value of 3.31 μg ergosterol per mg mycelial dry wt. Values present are means ± standard deviation ($n=30$)

zal colonization, visual indices derived by dividing the product of colonization percentage and morphological rating by 100 for each individual seedling were used. The visual indices exhibited no seasonal pattern (Table 4). The r^2 values between visual estimates of mycorrhizal colonization and total ergosterol level for seedlings sampled in July and August were 0.91 and 0.78, respectively. After August, r^2 was less than 0.5.

Seasonal dry weight partitioning of microplot-grown loblolly pine seedlings

Seedlings that were sampled for both chemical and visual assessments of mycorrhizal development were included in data presented in Figures 5a, b. Generally, variation was approximately 30% of the means for the biomass data at each sampling. With only 30 seedlings randomly sampled each time, this range of variation was not unexpected. Needle dry wt. increased the most from July through September (Fig. 5a). Stem and branch dry wt. increased the most during spring and the months of October and November. Tap roots and lateral roots accumulated weight through the fall (Fig. 5b). Similar to stems and branches, tap roots also in-

Table 3 Seasonal soil ergosterol levels and estimated ectomycorrhizal mycelial dry wt. in loblolly pine seedling microplots. It was assumed that only ectomycorrhizal mycelia were present in the soil of the loblolly pine microplots, with a content of 3.31 μg ergosterol/mg mycelial dry wt., and that the root systems of all 100 pine seedlings occupied the same amount of soil space in a microplot. Data shown are means ± standard deviation ($n=6$)

	Ergosterol (μg/l soil)	Estimated ectomycorrhizal mycelial dry wt.	
		mg/l soil	mg/seedling
July	149± 32.7	45.0	40.5
August	222± 51.6	67.1	60.4
September	314± 47.6	94.9	85.4
October	396±157.3	119.6	107.6
November	279± 79.5	84.3	75.9
December	393±196.9	118.7	106.8
February	417±218.1	126.0	113.4
March	247± 81.2	74.6	67.1
May	750±145.2	226.6	203.9

Table 4 Visual assessment of seasonal ectomycorrhizal development on loblolly pine seedling and its correlations with ergosterol analysis. The % root colonized values are the means of 30 seedlings. The visual indices are derived from (% short root colonized × morphological rating)/100 for each individual seedling and the numbers presented are the means of 30 values. r^2 is the correlation between visual index and total seedling ergosterol

Month	Short root colonized (%)	Morphological type rating	Visual index	r^2
July	23	2.57	0.94	0.912
August	51	3.39	1.89	0.785
September	54	4.05	2.37	0.472
October	62	5.36	3.57	0.411
November	50	3.80	2.07	0.328
December	45	4.45	2.10	0.155
February	88	3.10	2.65	0.456
March	92	3.30	3.04	0.233
May	95	3.85	3.66	0.325

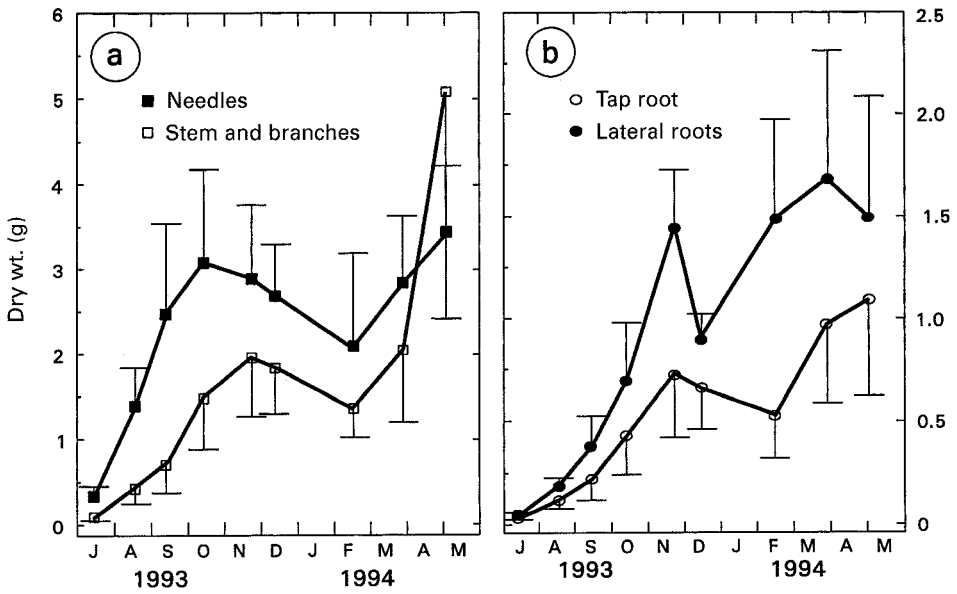

Fig. 5 Seasonal dry wt. partitioning patterns for **a** needles (■) and stem and branches (□), and **b** tap root (○) and lateral roots (●) of loblolly pine seedlings. Data were from the same 30 seedlings used for ergosterol analysis in Figure 4. Values presented are means ± standard deviation ($n=30$)

creased dry wt. in spring. During winter, all seedling parts appeared to exhibit weight loss.

Discussion

The ergosterol contents of all ectomycorrhizal fungi tested ranged between 1.0 and 8.0 μg/mg mycelial dry wt. and fell within the ranges reported in the literature (Salmanowicz and Nylund 1988; Martin et al. 1990; Antibus and Sinsabaugh 1993). Our data showed close correlations between total ergosterol levels and mycelial dry wt. for Tt, Rh, and Pt and verified the feasibility of estimating ectomycorrhizal fungal biomass by ergosterol analysis. However, age-related variation in ergosterol content should not be ignored when interpreting the results of fungal biomass derived from ergosterol analysis. Although variability in ergosterol content for Tt and Rh over time was low (Figs. 1c, 2c), threefold differences in Pt ergosterol content within an isolate were observed (Fig. 3c). Johnson and McGill (1990b) reported a temporal study of *H. crustuliniforme* that ranged from 1.5 to 4.9 μg ergosterol/mg mycelium between day 2 and 50 of incubation. When the data of Johnson and McGill (1990b) are plotted, an ergosterol content versus age curve similar to our data for Pt (Fig. 3c) is produced.

Antibus and Sinsabaugh (1993) found that the ergosterol content of the slow-growing species *Suillus tomentosus* (Kauff.) Sing, Snell & Dick was sixfold higher than that of the fast-growing species *Amanita rubescens* (Pers. ex Fr.) S. F. Gray. We did not observe differences of this magnitude in this study. For example, mycelial dry wt. of Rl, the fastest growing species, was six times greater than Tt, the slowest growing species (Table 1). However, the ergosterol content of Rl was only about twice as high as Tt. Of three species with similar dry wt., Cg has 50% less ergosterol than Hc or Sl. Moreover, there were less than onefold differences in ergosterol content over time between fast- and slow-growing cultures of Pt throughout the duration of the experiment.

Explanations of observed patterns of ergosterol content in nursery-grown loblolly pine seedlings are based on source-sink relationships and seasonal growth characteristics. Johnson and McGill (1990a) reported a temporal study of ergosterol content and mycelial dry wt. in ectomycorrhizal *Pinus contorta* seedlings. The percentage of mycelial dry wt. over *P. contorta* root dry wt. decreased from 12% in April to 2% in June and then increased to 9.7% in November (Fig. 3 in Johnson and McGill 1990a). A similar trend was observed in our study. However, increases and decreases in mycelial over root dry wt. occurred at different times of the year in the current study. In July, when root systems are small and the major carbon sinks in loblolly pine seedlings are stems and elongating needles (Fig. 5; Sung et al. 1993), 9% of the lateral root dry wt. was estimated to represent ectomycorrhizal fungal biomass (Fig. 4d). Through the growing season, both roots and mycelia increased in biomass; however, the percentages of mycelia dry wt. decreased because lateral roots were more active in dry wt. accumulation than mycelia (Fig. 4 vs Fig. 5). Sung et al. (1993) noted that toward late November and early December loblolly pine seedling roots became the sole sucrose sink until the following spring. In our study, total seedling ergosterol content continued to increase during winter but lateral roots and seedling tops actually lost weight. As a result, mycelial dry wt. percentages during winter months increased. Söderström and Read (1987) suggested that ectomycorrhizal mycelia depend on current photosynthate for respiration and growth. Consequently, both roots and associated mycorrhizal fungi of the seedlings were apparently the major carbon sinks in late fall and winter.

Using chitin analysis, Rousseau and Reid (1991) reported a range of 11% to 23% of mycelial dry wt. over

total root dry wt. during a period of 12 weeks after Pt inoculation of growth-chamber grown loblolly pine seedlings. One reason for the high values of mycorrhizal mycelial biomass in the study by Rousseau and Reid (1991) was the presence of chitin in both live and dead mycelia. Nonetheless, the results of this study are similar to those of Rousseau and Reid. Eight weeks after Pt inoculation, percentage of mycelial biomass started to decrease because root dry wt. increased faster than mycelial dry wt. (Rousseau and Reid 1991).

Based upon the ergosterol analysis, live fungal mycelia comprised nearly 4% of seedling lateral root dry wt. during the winter. The current study did not examine other potential carbon sinks, such as root and fungal respiration and fine root turnover. Nevertheless, for two reasons this study does suggest that mycorrhizal fungi import more carbohydrate than their weight indicates. First, if the analyzed soil ergosterol completely came from the extramatrical ectomycorrhizal mycelia left in the soil during harvest, these extramatrical mycelia and those from mycorrhizae accounted for at least 10% of lateral root weight. If a lesser portion of the soil ergosterol came from extramatrical ectomycorrhizal mycelia, the percentage weight of total ectomycorrhizal mycelia would still be greater than 4%. Second, extramatrical mycorrhizal mycelia respire 38% more than mycorrhizal roots, which respire almost twice as much as nonmycorrhizal roots (Rygiewicz and Andersen 1994). Söderström and Read (1987) found that nearly 30% of the total root system respiration (roots plus mycorrhizae plus extramatrical mycelia) of *P. sylvestris* and *P. contorta* seedlings colonized by several ectomycorrhizal fungal species could be attributed to extramatrical mycelia. High respiration rates of mycorrhizal fungal mycelia in proportion to roots would result in underestimates of carbon sink strength contributed by ectomycorrhizae.

In the spring, active elongating shoots and reactivated stem cambial tissues are the major sucrose sinks and roots become less active sinks (Fig. 5; Sung et al. 1993). The mycorrhizal mycelial network already in the soil would be responsible for soil nutrient and water uptake to meet the growth demands of the aboveground parts. This phenomenon has been suggested by Jones et al. (1991) on the carbon cost of phosphorus uptake by ectomycorrhizae. In loblolly pine, this may be the physiological reason for maintaining a carbon-costly mycorrhizal symbiont during winter when the supply of current photosynthate is low.

The correlation between visual and chemical assessment of mycorrhizal colonization that existed early in the growing season, when seedlings were small and had fewer lateral roots, disappeared later in the growing season. The large variation in total ergosterol level among seedlings at each sampling period indicated that seedlings were not synchronized relative to mycorrhizal development. Variation in colonization between individual lateral roots of a seedling made visual assessment useless unless each lateral root was carefully assessed for morphological types, percentage of short root colonization, total number of colonized short roots per lateral root, and number of total lateral roots per seedling. In addition, variation in thickness of the mycorrhizal mantle could hinder visual assessment of mycorrhizal colonization to estimate fungal biomass. The problems associated with visual assessment make ergosterol analysis an attractive alternative.

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